

Remarks

Claims 1-7, 10-11, 13, 18-19, 23, 28, 31 and 33-37 are currently pending and under examination. Claim 1 is amended. No new matter is added.

Applicants respectfully request withdrawal of the finality of the rejection. The Office Action raises a new ground of rejection based on art not previously cited. As set forth in MPEP 706.07(a), "second or any subsequent actions on the merits shall be final, **except** where the examiner introduces a new ground of rejection that is neither necessitated by applicant's amendment of the claims nor based on information submitted in an information disclosure statement filed during the period set forth in 37 CFR 1.97(c) with the fee set forth in 37 CFR 1.17(p)." Applicants submit that Veres and Stadtman (1994) is newly cited by the Examiner, and is applied to Claim 33, which has not been amended in prosecution. The finality of the Office Action is therefore improper.

Specifically, Applicants have provided references to support Applicants position that the present claims are patentable in view of the newly cited art. As Applicants could not reasonably have provided these references earlier in prosecution, entry is requested. Applicants have also made an amendment to Claim 1, clarifying the subject matter of the claim. Entry of the amendment is requested.

Claims 1 and 33 have been rejected under 35 U.S.C. 102 as anticipated by Veres and Stadtman (1994). Applicants respectfully submit that the cited reference does not anticipate the presently claimed invention.

The Office Action states that Veres and Stadtman (1994) teach a method of biosynthetically labeling RNA in a cell of interest, the method comprising contacting the cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA, wherein the cell comprises a phosphoribosyltransferase or nucleoside kinase or phosphorylase that can specifically incorporate the purine or pyrimidine analog into the corresponding nucleotide.

The Office action further states that "since 2 thio-uridine is not normally a component of RNA therefore it is inherently obvious that the strain of E. coli that can produce the thiolated tRNA contains the enzymes necessary to convert the purine or pyrimidine analogs into RNA containing 2-thiouridine."

Applicants respectfully submit that this is factually incorrect. The methods taught by Veres and Stadtman utilizes naturally occurring thiolated tRNAs, because 2-thiouridine residues are naturally found in bacterial tRNAs. As evidenced by Rogers *et al.* (1995) Biochimie 77:66-74 (copy attached), "the first position or "wobble" base in the anticodon of tRNAs is frequently the site of post-transcriptional

modification. In *Escherichia coli*, glutamine, glutamate, and lysine tRNAs contain 2-thiouridine derivatives in this position." Therefore, there is **no** purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell in the methods of Veres and Stadtman.

Applicants further submit that Veres and Stadtman do **not** contact a cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell, as is set forth in the presently claimed invention.

The Materials and Methods of Veres and Stadtman states that:

Baker's yeast tRNA and *Escherichia coli* bulk tRNA were from Boehringer Mannheim. *E. coli* tRNA^{Glu} was from Sigma. Bulk tRNA from wild-type *S. typhimurium* cells grown in the presence of 1 μ M selenite (+Se) and bulk tRNA from wild-type *S. typhimurium* cells grown in the absence of added selenite (–Se) were isolated as described (5, 7). Bulk tRNA lacking 5-methylaminomethyl-2-thiouridine was isolated from cells of an *E. coli* *asuE* mutant strain (11) unable to synthesize 2-thiouridines. Periodate-oxidized tRNAs were

Therefore, the *E. coli* and yeast tRNA were purchased from commercial sources, and the cells were not contacted with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell.

The *S. typhimurium* tRNA was obtained from cells grown according to the procedure set forth in endnote 7, which is a publication by the authors, Veres et al. (1990) PNAS 87:6341-6344. With respect to the growth of bacteria, the authors state:

Growth of Bacteria. *S. typhimurium* was cultured anaerobically in Luria broth/0.5% glucose/20 mM potassium phosphate, pH 7.0, in the absence of added selenium. Cells were labeled with ⁷⁵Se during anaerobic growth at 30°C in a minimal medium consisting of Vogel–Bonner salts (27)/0.5% glucose/3.7 μ M FeCl₃/1 μ M ⁷⁵SeO₃²⁻ (2 mCi/liter; 1 Ci = 37 GBq).

Therefore, the *S. typhimurium* cells were not contacted with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell.

The Examiner particularly notes the *E. coli* *asuE* mutant strain which is unable to synthesize 2-thiouridines. Applicants fail to see the relevance of this strain with respect to the present invention. In the methods of Veres and Stadtman, the *asuE* strain was used to produce tRNA lacking 2-thiouracil. This strain of *E. coli* was not contacted with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell, nor did this strain of *E. coli* incorporate a purine or pyrimidine analog into RNA.

The Examiner also notes page 8094, last paragraph of the cited reference. Again, Applicants fail to see the relevance of this teaching to the methods of the present invention. The cited paragraphs read as follows:

Bulk thio-tRNA preparations from *E. coli* and wild-type *S. typhimurium* were equally effective as substrate (3, 11) for

the selenium incorporation reaction catalyzed by the purified *Salmonella* enzyme (Table 7). As expected, bulk tRNA isolated from the *E. coli* *asuE* mutant strain, which lacks 2-thiouridine, did not serve as substrate. Also, bulk tRNAs isolated from wild-type *S. typhimurium* grown in the presence of added selenite could not be further modified. *E. coli* tRNAs containing oxidized 3'-adenosyl groups (formed by treating deacylated tRNAs with periodate) were equally effective as substrate, showing that an intact 3' end of the molecule is not essential for selenium incorporation. Although lysine and glutamate tRNAs of baker's yeast contain 2-thiouridines in the wobble position of their anticodons (14), no selenium was incorporated into the commercial preparation tested. Whether this is due to the presence of the methoxycarbonylmethyl substituent at position 5 of the 2-thiouridine residue in these tRNAs is not known.

The authors essentially state that tRNA that is "store bought" or from wild-type *S. typhimurium* is a substrate in cell-free reactions for selenium incorporation, while the mutant tRNA lacking 2-thiouridine is not a substrate for the enzyme.

If the section is cited in order to teach the labeling of mRNA, Applicants respectfully submit that the cited art does not teach "conjugating a tag to said reactive moiety", as set forth in the present claims. In the prior art methods, a 2-thiouridine residue is converted to 2-selenouridine by replacement of the sulfur with selenium, not by conjugation of a tag to a reactive moiety.

Applicants respectfully submit that the cited art fails to teach a method wherein cells are contacted with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell, and as such cannot anticipate the present invention. Withdrawal of the rejection is requested.

Claims 1-7, 10-11, 13, 18-19, 23-28, and 31 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Veres and Stadtman (1994) Proc. Natl. Acad. Sci. USA vol. 91, pp. 8092-8096 as applied to claim 1 above in view of Trudeau et al. (2001) Human Gene Therapy 12: 1673-1680; and further in-view of Rana P.G. Pub 2004/0175732 filed on November 17, 2003 with a priority date of November 15, 2002.

Applicants respectfully submit that the present claims are not taught or suggested by the cited combination of art. The analysis of Veres and Stadtman is as described above – the reference fails to teach contacting a cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell, and since no analog was provided, it cannot be incorporated into RNA.

With respect to Claims 2, 3, 4, 23, 28 and 31, Trudeau is cited for teaching a genetic construct where HGPRT is cloned into a retroviral vector. The secondary reference does not remedy the deficiencies of the primary reference. While the reference teaches the cloning of an enzyme in various permutations, it does not teach a method of contacting a cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell, where the analog is incorporated into the corresponding nucleotide and wherein the analog is incorporated into RNA comprising the reactive moiety.

Applicants do not dispute that a wide variety of phosphoribosyltransferases, nucleoside kinases and phosphorylases have been cloned and expressed from a wide variety of vectors. However, it is the specific use of the enzymes in Applicants' methods that is claimed, and the sum of the references does not provide essential features of the present invention.

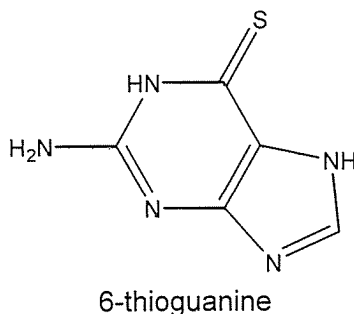
With respect to Claims 1, 6, 7, 10, 11, 13, 18, 19, Rana *et al.* is cited for teaching labeling, amplification and isolation of mRNA. The secondary reference does not remedy the deficiencies of the primary reference. While the reference teaches labeling of RNA, it does not teach a method of contacting a cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell, where the analog is incorporated into the corresponding nucleotide and wherein the analog is incorporated into RNA comprising the reactive moiety.

Applicants do not dispute that a wide variety of labels and method of affinity chromatography and amplification are known in the art. However, it is the specific use of the enzymes in Applicants' methods that is claimed, and the sum of the references does not provide essential features of the present invention.

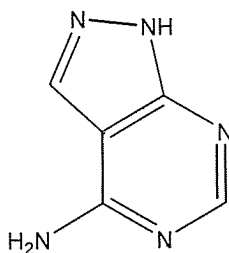
The Office Action states that Trudeau *et al.* "*teach the entire method and explain the principle in detail* how parasite encoded phosphoribosyltransferase enzyme can be used to incorporate purine analogs into newly synthesized RNA but do not enunciate how this labeled RNA can be isolated from the cell."

Applicants submit that this characterization of Trudeau *et al.* is incorrect. The reference does not teach Applicants methods or explain the principle in detail. As discussed in Applicant's previous

response, Trudeau teaches the introduction of a guanine analog having a structure as shown below, where there is a reactive group at the 6 position.



This reactive group is not incorporated into mRNA. The analog is converted into APPR-MP. The structure for this compound is as follows:



4-aminopyrazolo[3,4-d]pyrimidine

It can be readily observed that the reactive group is no longer present, and thus is not incorporated into the host cell RNA, and cannot provide a reactive moiety not normally present in nucleic acids.

Trudeau is not directed to biosynthetically labeling RNA in a cell, but rather to killing a cell (e.g., a lung cancer cell) by sensitizing the cell to allopurinol. The stated use for this method is as a therapeutic tool for gene prodrug targeting of lung cancer. See the Abstract. Hence, Trudeau is not concerned with producing thio-labeled RNA but rather to producing cytotoxic metabolites that induce apoptosis in a cell. Once the cell has been killed, Trudeau has no use for any of the remaining cellular components. Accordingly, Trudeau neither teaches nor suggests either obtaining RNA from the killed cells and/or conjugating a tag to the RNA obtained. One of skill in the art would not be motivated to modify Trudeau such that after the cells are killed RNA from the killed cells is obtained and conjugated with a tag, because to do so, even if possible, would serve no purpose and would therefore be to change Trudeau's principle of operation in contravention of M.P.E.P. § 2143.01.

In view of the above remarks, Applicants respectfully request withdrawal of the rejection.

Claims 33-35 and 37 have been rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Veres and Stadtman (1994) Proc. Natl. Acad. Sci. USA vol. 91, pp. 8092-8096, in view of Al-Anouti et al. (January 2003) Biochemical and Biophysical Research Communications vol. 302: pp. 316-323.

Applicants respectfully submit that the present claims are not taught or suggested by the cited combination of art. The analysis of Veres and Stadtman is as described above – the reference fails to teach contacting a cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell, and since no analog was provided, it cannot be incorporated into RNA.

Al-Anouti is cited as a secondary reference against Claims 33-35 and 37. The secondary reference does not remedy the deficiencies of the primary reference. While the reference teaches methods relating to uracil phosphoribosyltransferase, it does not teach a method of contacting a cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell, where the analog is incorporated into the corresponding nucleotide and wherein the analog is incorporated into RNA comprising the reactive moiety.

Al-Anouti is directed to a method of modulating gene expression of *T. gondii* using dsRNA. In accordance with the methods disclosed by Al-Anouti, a *T. gondii* parasite is transfected with a vector containing the genetic sequence for UPRT, from which dsRNA homologous to UPRT is produced. The dsRNA is degraded into small interfering RNA (siRNA) which then interferes with the UPRT gene so as to down-regulate UPRT gene expression, resulting in decreased UPRT activity. See page 323, 2nd full paragraph. Thus, Al-Anouti proposes a method for decreasing UPRT activity, not for utilizing UPRT activity in the biosynthetic labeling of RNA.

Al-Anouti do not show that a purine or pyrimidine analog can be incorporated into RNA. The reference teaches the use 5-fluoro-2-deoxyuridine (FDUR) for its known toxicity to cells expressing UPRT. However, this toxicity occurs via inhibition of thymidylate synthase (essential for DNA synthesis) by the 5-fUMP produced by the UPRT. There is no evidence in this paper that the 5-fUMP is incorporated into RNA. This does show how UPRT converts a uracil analog to the corresponding uridine monophosphate but not how it converts it to a form that can be used to label RNA.

The engineered expression of UPRT in this reference is designed to express a double stranded RNA construct, not a functional UPRT enzyme. The assertion that the authors designed a plasmid to express UPRT in bacteria and human foreskin fibroblast (HFFs) is not correct. The growth in bacteria is simply for generating plasmid DNA and the construct is never put into HFF cells, the HFF cells are hosts for Toxoplasma that are transfected with the plasmid. There is no transgenic expression of UPRT in an organism other than Toxoplasma in this paper.

Applicants do not dispute that a wide variety of phosphoribosyltransferases, nucleoside kinases and phosphorylases have been cloned and expressed from a wide variety of vectors. However, it is the specific use of the enzymes in Applicants' methods that is claimed, and the sum of the references does not provide essential features of the present invention.

In view of the above remarks, withdrawal of the rejection is requested.

Claim 36 has been rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Veres and Stadtman (1994) Proc. Natl. Acad. Sci. USA vol. 91, pp. 8092-8096, in view of Al-Anouti et al. (January 2003) Biochemical and Biophysical Research Communications vol. 302: pp. 316-323 as applied in claims 33-35 and 37 above further in view of Iltzsch and Tankersley (1994) Biochem Pharm. Vol. 48 (4): 781-792 cited by applicant in IDS.

Applicants respectfully submit that the present claims are not taught or suggested by the cited combination of art. The analysis of Veres and Stadtman, and of Al-Anouti is as described above.

The secondary reference does not remedy the deficiencies of the primary reference. While the reference teaches methods relating to 2,4-thiouracil, it does not teach a method of contacting a cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA of that cell, where the analog is incorporated into the corresponding nucleotide and wherein the analog is incorporated into RNA comprising the reactive moiety.

The Office Action states that Iltzsch and Tankersley are cited for teaching that 2,4 thiouracil is a substrate for the *T gondii* enzyme UPRT, and thus 2,4 dithiouracil could be incorporated into RNA using the UPRT enzyme present in the bacterial cell and thiollated RNA can be isolated from such a cell which in turn can be used to conjugate with desired label using the available thiol group in the RNA as a reactive moiety.

Applicants respectfully submit that Iltzsch and Tankersley fail to teach methods wherein cells are contacted with a purine or pyrimidine analog. All of the work identifying UPRT substrates by Iltzsch and Tankersley were performed using parasite lysates, not living organisms outside the animal. There is no guarantee that a live cell will efficiently take up a given substrate, let alone that that will be a substrate for subsequent steps in the pathway (i.e., the kinase that takes UMP to UTP or the RNA polymerase that incorporates that into RNA) or be bioavailable in an animal.

There is also no evidence in Iltzsch and Tankersley that any of the uridine monophosphate forms of these analogs will be substrates for RNA polymerase and end up incorporated into RNA. So there is no evidence in this paper to show that addition of uracil analogs to whole cells will result in labeling of RNA.

Applicants have previously provided an article by Pfefferkorn *et al.* (2001), which describes, similarly to Trudeau *et al.*, the activity of thiolated purine analogs in *Toxoplasma gondii*. Importantly, the authors find that the analogs are not incorporated into the host DNA, but rather act to inhibit synthesis of guanine nucleotides by blocking dehydrogenase, thus teaching away from the present invention.

Pfefferkorn shows that, whereas one of ordinary skill in the art might have assumed that 6-thioxanthine was toxic because it gets incorporated into 6-thioXMP and thence into 6-thioGMP and RNA or DNA, that is in fact not what happens. This compound is recognized by *Toxoplasma* HXGPRT and converted to 6-thioXMP but it is not further metabolized - it is toxic in that form. Hence, it is not at all a given that just because a thiobase is utilized by a phosphoribosyl transferase that it will be incorporated into RNA.

Applicants do not dispute that 2,4-thiouracil was known in the art. However, it is the specific use of the enzymes in Applicants' methods that is claimed, and the sum of the references does not provide essential features of the present invention.

In view of the above remarks, withdrawal of the rejection is requested.

Conclusion

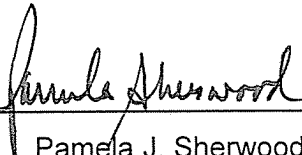
Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number STAN-304.

Respectfully submitted,

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Date: July 16, 2007

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